Um-Salama Science Journal **Vol.6(2)2009**

Spectrophotometry of Saliva of Oral Squamous Cell Carcinoma Patients

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Date of acceptance 28 /12 / 2008

Abstract:

Back ground: Spectral analyses of solutions have long been applied to various body fluids for the purpose of clinical study as well as research .Human saliva can be easily obtained by non invasive .In this study typical spectra (for UV and IR) of saliva of oral cancer Squamous cell carcinoma patients were determined under average conditions and evaluated in relation to the spectra of normal specimens.

Materials and Methods: Seventeen patients of oral cancer Squamous cell carcinoma and seventeen age matched healthy subject were included in this study .Chewing - Stimulated Saliva was collected in plastic test tube and stored at -20° C. Bach of saliva samples were used for UV and IR measurements.

Results: Many differences between the IR spectrum of saliva of oral Squamous cell carcinoma patients and IR spectrum of normal saliva.

Conclusion: The results in this study were detected that the use of IR spectroscopy may be useful in the diagnosis of oral Squamous cell carcinoma by using saliva samples.

Key words: Spectrophotometry, Saliva, Oral Cancer.

Introduction:

The vast majority of oral cancers are of epithelial origin, developing from the lining tissues of the oral cavity; hence, about 90% of the oral cancer seen by dentists well is Squamous cell carcinoma. (1.2)

Of all the organs in the craniofacial-oral-dental complex, it is perhaps the salivary glands and their remarkable secretors product, saliva that forage the strongest link between oral well being, ranging from subtle effects of over-the counter cold medications to the devastation of life-threatening disease. Research has found anew role for saliva as an effective laboratory tool. (3).

Long known primarily for its protective and lubricating properties, saliva is now meeting the demand for inexpensive, non invasive and easy-to-use diagnostic aids for oral and systemic diseases, and for assessing risk behaviors such as tobacco and alcohol use. (4, 5)

Spectral analyses of solutions have long been applied to various body fluids for the purpose of clinical study as well as research. UV light absorbance measurements were made for many purposes: to determine the concentration of substance, to assay certain chemical reactions, to identify materials, and to determine the structural parameters of macrbmolecules. (6)

Although the analysis of biological tluids has along tradition in providing information to suggest or corroborate diagnosis, complementary technique is emerging for the interpretation of

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the IR spectra. Rather than deriving analyze levels explicitly from them, the spectra may be viewed as finger prints that correlate directly with the presence or absence of disease. Because the spectra are complex, patterns characteristics of specific diseases are rarely (if ever) discernable from visual examination of the spectra. However, multivariate analytical methods may identify subtle patterns distinguishing the spectra corresponding to "normal" specimens from those corresponding to diseased patients. (7, 8)

The purpose of this study was determined the typical spectra (UV and IR) saliva of oral squamous cell carcinoma patients .under average conditions and evaluated in relation to the spectra of normal (control) specimens.

Materials and Methods:

Seventeen patients of oral squamous carcinoma were included in this study. In addition seventeen ages matched healthy subject were also included. All patients were admitted for treatment to hospital, and AL-Karkh Emergency hospital. They were histological proven, newly diagnosed and not under went any type of therapy. Patients suffered from any disease that may interfere with our study were excluded. Ten males and seven females with oral squamous cell carcinoma, their age range between 21-60 years were investigated.

Chewing-stimulated saliva was collected in plastic test tube and stored at -20 C. Before use. it was thawed and centrifuged (lOmin at 1500xg) and the supernatant liquid was used for UV analyses. Bach of saliva samples were diluted (1:6) by deionizer water and measured at the wavelengths from 200 to 300nm by shimadzu 160-UV-visible record spectrophotometer.

Infrared (IR) absorption spectra of saliva were obtained by using dried saliva films, as batch of saliva samples were lyophilized and then measured by Perkin-Elmer infracord spectrophotometer.

Results:

Saliva samples of oral cancer patients and normal individuals were studied by using UV light. Figure (1-A, B) show's that saliva samples have the same maximum wave length *(k* max.) at 214nm, for both oral cancer patients and normal individuals. Another peak of absorbance at 274nm. For saliva sample of OSCC patients and at 282.5nm. For normal saliva sample. In general, UV absorption spectrum of saliva of oral cancer patients has the same form of the spectrum of normal saliva.

The IR spectrum of the dried film of saliva samples of oral cancer patients and normal individuals are presented in figure (2), between 4000 cnri and 700cnri. The wave numbers of the main absorption as well as their intensities are summarized in table (1). From these results many differences between the spectrum of oral cancer patients and normal saliva were detected:

1-The band at 1500cnri appeared as a shoulder in normal saliva infrared spectrum, while it disappeared in the spectrum of saliva of oral cancer patients.

2-The strong band of normal saliva at (1425-1450)cnri appeared as abroad in the case of oral cancer patients' spectrum. 3-The band (1170-980)cnri appeared as a strong and broad in normal saliva and saliva of oral cancer patients respectively. 4-The band (900-930)cnri appeared as a strong band in saliva samples from oral cancer patients' infrared spectrum,

while it disappeared in the spectrum of normal saliva.

Table (1): Infrared were numbers and intensities of saliva samples infrared bands.

Normal Saliva		Saliva samples from oral cancer patients	
Wave number (cm^{-1})	Intensity	Wave number (cm^{-1})	Intensity
3300-2800	S	3300-2800	S
2200-2000	W	2200-2000	W
1650-1550	S	1650-1550	S
1500	Sh		
1425-1450	S	1290-1450	Broad
1170-980	S	1170-980	Broad
—		900-930	S
830-700	broad	_	

Abbreviations = S: Strong, W: Weak, Sh:Shoulder.

Discussion:

Because it is so readily available, saliva has often been considered as a potential source of diagnostic information (5). Diagnostic biomarkers in saliva have been identified for monitoring caries, periodontitis, oral cancer, salivary gland diseases, and systemic disorders, e.g., hepatitis and HIV(9). Saliva is characteristically a colorless dilute fluid, with a density ranging from 18 to 35. lts pH is usually around 6.64, although a variety of components is always present in saliva, the total concentration of inorganic and organic constituents is generally low when compared to serum. Specific proteins, such as the enzyme amylase, are synthesized in the salivary glands and may be present in saliva in concentrations exceeding those of serum. Other organic components existing in saliva include: maltase, serum albumen, urea, uric acid, creatinine, mucine, vitamin C, several amino acids, lysozime, lactate, and some hormones such as testosterone and Cortisol. Saliva contains immunoglobins such as IgA and IgG, at an average concentration of 9.4 and 0.32mg%, respectively (10-14).

Most of biological macromolecules absorb ultraviolet (UV) tight in a range of wavelengths that is easily measurable, as a result of their containing aromatic rings. The absorption spectra of some of the amino acids have been well studied and are of great use both in identifying substances and in determining the structure of proteins (15). Form results in Fig (1) it is observe that the peaks of absorbance were approximately similar in wavelengths and there was a little difference in the value of absorbance as it was more in the case of normal saliva than saliva of oral cancer patients, which may reflects a different level of proteins.

Infrared (IR) spectroscopy has emerged in recent years as the analytical method of choice in an enormous variety of applications. The general procedure for developing this diagnostic test has much in common with the techniques employed to develop IR-based analytical methods. The first step's to acquire appropriate specimens from two sets of donors. One set of normal or control samples is required, whereas the second set corresponds to patients who have been diagnosed by traditional methods as having the disease of interest.

Infrared spectroscopy in the 4000-700cm^{-1} region was used to characterize the saliva constituents for normal individuals and oral cancer patients. From the results in Fig (2) many differences between the spectrum of oral cancer patients and normal saliva were detected (Tabic: 1). The lipid constituents provide

clear absorptions in the IR spectra, the spectral region 2800-3300cm^{-1}(16), showed a strong peak in both the saliva of oral cancer patients and normal saliva. The region 2200-2000cm^{-1} was appeared as a weak band in both normal and pathological samples. The absorption at 2060cm^{-1} is form endogenous thiocyanate(SCN^{-}). Although it is somewhat surprising to learn that this ion is present in appreciable amounts in human saliva, it plays a functional role; enzymatic conversion yields salivary hypothiocyanate (OSCN-), which is a highly effective antibacterial agent (17).

The IR spectrum of the dried film (Fig.2), of saliva of oral Squamous cell carcinoma and normal saliva, reveals not only lipid and SCN^{-} ion but also protein constituents. The bands 1650-1550cm^{-1} (s), 1500 cm^{-1} (sh.), and 1425-1450cm^{-1} (s) of saliva of oral cancer patients, have been explored in attempting to quantitate saliva protein (16. 17). In all region spectrum the absorbance of the saliva of oral cancer patients more than that of normal saliva, except in the 1450-1550cm^{-1} region which a protein absorbance region and this is agree with the results of UV spectroscopy in this study. Another main difference was disappeared of the peaks 1500cm^{-1} (sh.) and 1425-1450cm^{-1} (s) in the spectrum of saliva from oral cancer patients compared with the spectrum of saliva from normal individuals. That result indicated there were aerial difference in protein levels between the saliva of oral Squamous cell carcinoma and saliva of normal persons, and may be not in both samples.

Glucose provide clear absorption in the IR spectra, the region 1170-980 cm^{-1} (18, 19), showed a strong peak in the dried film of saliva of normal individuals and broad in the case of oral cancer patients. In conclusion, the use of IR spectroscopy may be useful in the diagnosis of oral Squamous cell carcinoma by using saliva samples. Saliva can be used as a diagnostic specimen not only to obtain information more inexpensively and efficiently than serum, but also to provide information not readily available from serum testing.

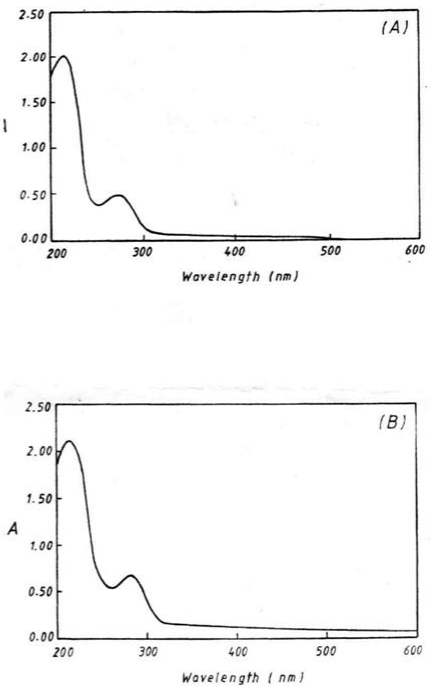

Fig. (1): A - Absorption spectrum of saliva of oral cancer patients.
B-Absorption spectrum of normal saliva.

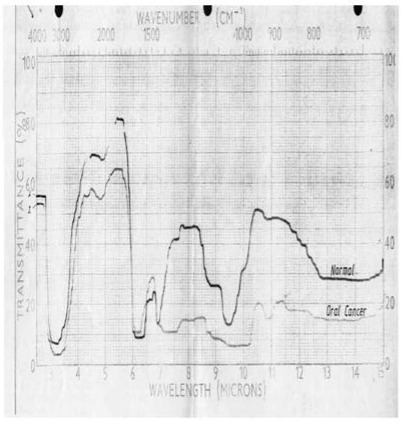

Fig (2): Infrared absorption Spectra of Saliva of Oral Cancer Patients and Saliva of normal individuals

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دراسة طيفية للعاب مرضى سرطان الخلايا الحرشفية للفم مقارنة بلعاب الأصحاء.Oral Squamous Cell Carcinoma

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الخلاصة:
تم تعيين أطياف الأشعة فوق البنفسجية (UV) والأشعة تحت الحمراء (IR) في هذه الدراسة لنماذج لعاب مرضى سرطان الفم Oral Squamous Cell Carcinoma ونماذج اللعاب للأصحاء. ومن ثم مقارنة النتائج لغرض الحصول على الخط الخاص لكل منها ومن ثم الإستفادة منه لإغراض الدراسة السريرية. عينت العديد من الفروق بين الأشعة تحت الحمراء (IR) للعاب مرضى سرطان الفم وطيف لعاب الأصحاء.
أقرت النتائج في هذه الدراسة إمكانية إستعمال مطيافية IR لأغراض التشخيص لمرض سرطان الفم باستخدام عينات اللعاب